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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	: Yoshiharu MATAHIRA et al.
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DECLARATION UNDER 37 CFR 1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS

WASHINGTON, D.C. 20231

SIR;

Now comes Yoshiharu MATAHIRA who deposes and says that:

1.I am a co-inventor of the invention described and claimed in the above-referenced application.

2.I graduated from Shizuoka University, Faculty of Agriculture, Department of Agricultural Chemistry in 1984, and received my doctoral degree in agriculture from Gifu University, United Graduate School, Agricultural Research Course in 1995, and has been employed by Yaizu Suisan Kagaku Industry Co., Ltd. since 1984.

3.Under my supervision and control, the following experiments were carried out:

TEST EXAMPLE 1 (Quantitative analysis of imidazole dipeptide in yeast extract)

14g of a yeast extract powder (manufactured by Yaizu Suisan Kagaku Industry Co., Ltd.) was dissolved in 10ml of trichloroacetic acid, and then this solution was filtrated by a filter of 0.20μm, and this filtrate was subjected to amino acid analysis by a Hitachi amino acid analyzer L-8500A, to quantitatively analyze the imidazole dipeptide in the powder.

Further, with respect to a sample prepared in the same manner as above, quantitative analysis of imidazole dipeptide was carried out in accordance with the HPLC method by OPA derivatization procedure on page 316, lines 10-40 of "J. Chromatogr., 342, 315-320, (1988)" (the copy of this document is attached).

The results are shown in the following table.

Table 1

	Anserine (mg/100mg)	Carnosine (mg/100mg)
Amino acid analysis	176.7	Not detected
OPA derivatization	Not detected	Not detected

In the imidazole peptide, anserine was detected by the amino acid analysis, but not detected by the OPA derivatization procedure. The detection limits of the amino acid analysis and the OPA derivatization are at the same level. The OPA derivatization exhibits a higher specificity to imidazole dipeptide than the amino acid analysis. From these facts, we consider that the result of the amino acid analysis is

pseudopositive. Accordingly, we conclude that no imidazole dipeptide is contained in the yeast extract powder.

TEST EXAMPLE 2(Influence of anserine on actomyosin ATPase activity of bonito muscle)

Actomyosin was prepared from fresh bonito normal muscle in accordance with the description of "Nissuishi, 39, 1077-1085 (1973)" which is summarized in the following (1) to (5).

Preparation of actomyosin:

(1)To fresh bonito normal muscle, a cold phosphoric acid buffer solution (ionic strength: 0.05, pH 7.5) of 4 times amount was added, followed by stirring. After centrifugation (3,000 g, 10 minutes), the supernatant is removed. This operation is repeated 3 or 4 times.

(2) To the bonito normal muscle after the step (1), 0.45M KCl-phosphoric acid buffer solution (pH 7.5) of 3 times amount was added. Then, extraction was carried out for 20 hours while sometimes stirring this solution.

(3) The extract was slowly poured with stirring into cold distilled water of 10 times amount, and then pH was adjusted to 6.5-6.6 with a 5% acetic acid. This solution is left in a cold site, and the precipitated protein is recovered by centrifugation (3,000 g, 10 minutes).

(4) 3M KCl is added to the precipitate to adjust to 0.6M KCl, to dissolve the precipitate.

(5) Using a dialysis tube, dialysis was carried out overnight in the 0.6M KCl solution, followed by centrifugation (20,000

g, 60 minutes). This supernatant is used as an actomyosin solution.

The prepared actomyosin was reacted at 30℃ in a buffer solution (pH 7.4) containing 2mM ATP, 20mM Tris-maleate and 20mM KCl. Inorganic phosphor deliberated with the lapse of time was qnantitatively analyzed to determine the ATPase activity [μ mol Pi/min/mg protein]. Further, to this reaction system, anserine was added so that it would be 10mM, and the ATPase activity was determined for the purpose of comparison.

The results are shown in the following table.

Table 2

	ATPase activity [μmol Pi/min/mg protein] (ave.±SD, n=3)
Anserine not added	0.087±0.013
Anserine added	0.111±0.005

The ATPase activity when anserine was added shows 30% higher numerical value than the ATPase activity when no anserine was added. This data suggests that anserine can activate the ATPase in the muscle.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 25th/June/2003

Yoshiharu MATAHIRA